



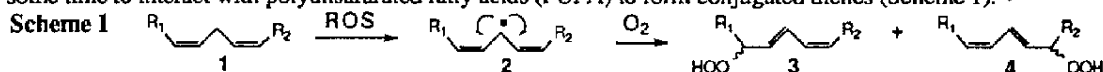
## Total Synthesis of a Novel Isoprostane IPF<sub>2α</sub>-I and Its Identification in Biological Fluids

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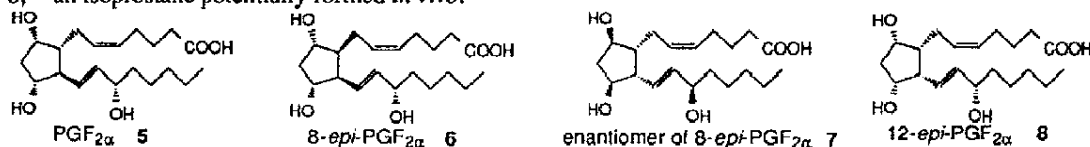
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**Abstract:** The first total synthesis of IPF<sub>2α</sub>-I **25** is described using D-glucose as starting material. This novel isoprostane has been used to establish its presence in human urine. Copyright © 1996 Elsevier Science Ltd

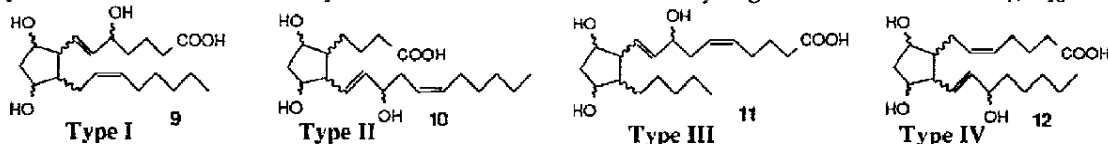
More and more we have come to the realization that, in addition to the necessary enzymatic machinery which keeps us alive, a parallel non-enzymatic free-radical-mediated biochemical system is operative.<sup>1</sup> Reactive oxygen species (ROS), such as  $\cdot\text{OH}$ ,  $\cdot\text{OOH}$ ,  $\text{ROO}\cdot$ ,  $\cdot\text{OO}\cdot$ , which are products and by-products of enzymatic reactions, and  $\text{CH}_3\cdot\text{CHOH}$  and  $\text{CH}_3\cdot\text{CO}$  and  $\cdot\text{CH}_3$ , by-products of alcohol consumption have been known for some time to interact with polyunsaturated fatty acids (PUFA) to form conjugated dienes (Scheme 1).<sup>1,2</sup>



This peroxidation process has been linked to pathological injury in diseases such as hepatorenal syndrome,<sup>3</sup> alcohol-induced liver disease,<sup>4</sup> pulmonary hypertension,<sup>5</sup> myocardial infarction<sup>6</sup> and atherosclerosis.<sup>7</sup> Recently 8-*epi*-PGF<sub>2α</sub> **6**, a member of a new class of products, the isoprostanes, has been identified in *in vitro* and *in vivo* systems as a product of free-radical peroxidation of arachidonic acid (AA) and has been shown to be a potent vasoconstrictor.<sup>8</sup> It has also been identified as a minor by-product in the enzymatic cyclooxygenase-1 (COX1)<sup>9</sup> and COX2<sup>10</sup> peroxidation of AA. We have performed the first total synthesis of 8-*epi*-PGF<sub>2α</sub> **6** and its enantiomer **7**,<sup>11</sup> and recently reported the total synthesis of 12-*epi*-PGF<sub>2α</sub> **8**,<sup>12</sup> an isoprostane potentially formed *in vivo*.

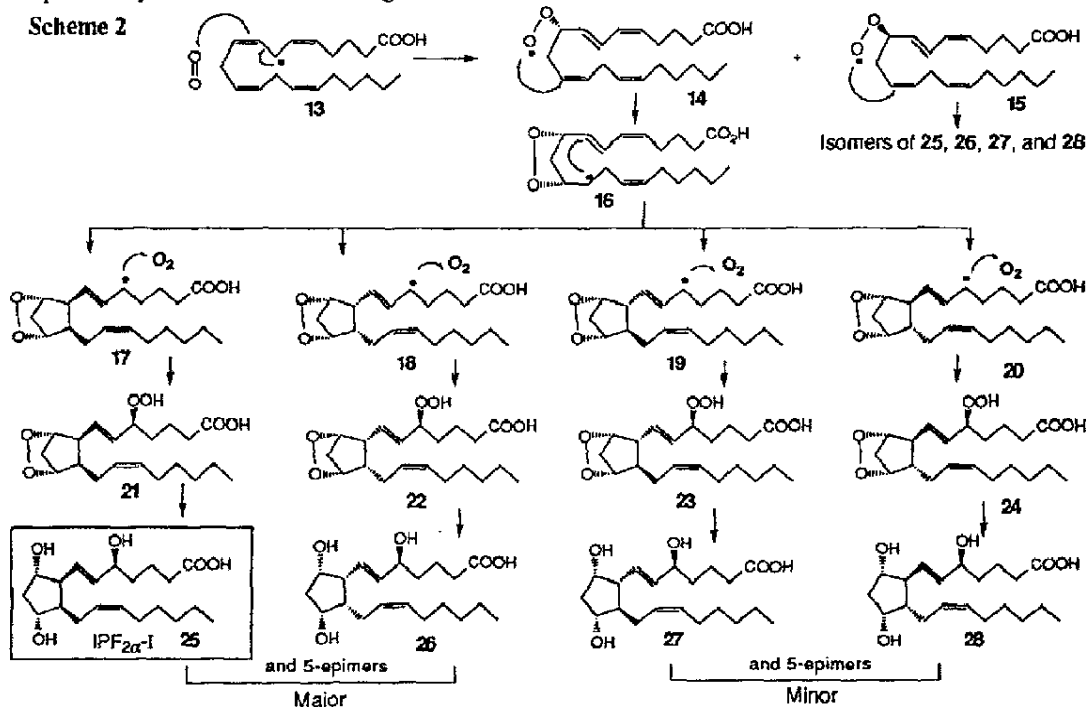


Four types of isoprostanes **9**, **10**, **11** and **12** have been proposed as potential products of ROS-initiated peroxidation of AA.<sup>11-13</sup> They are formed as a result of an initial hydrogen atom abstraction at C7, C10 and



C<sub>13</sub> of AA. Isoprostanes 6, 7, and 8 belong to Type IV isoprostanes.

We show in Scheme 2, a detailed analysis of the steps leading to the generation of isoprostanes of Type I from AA by a free-radical peroxidation process. The purpose of this exercise is to help us predict and evaluate the probability of which stereo- and regioisomers are formed *in vivo*.

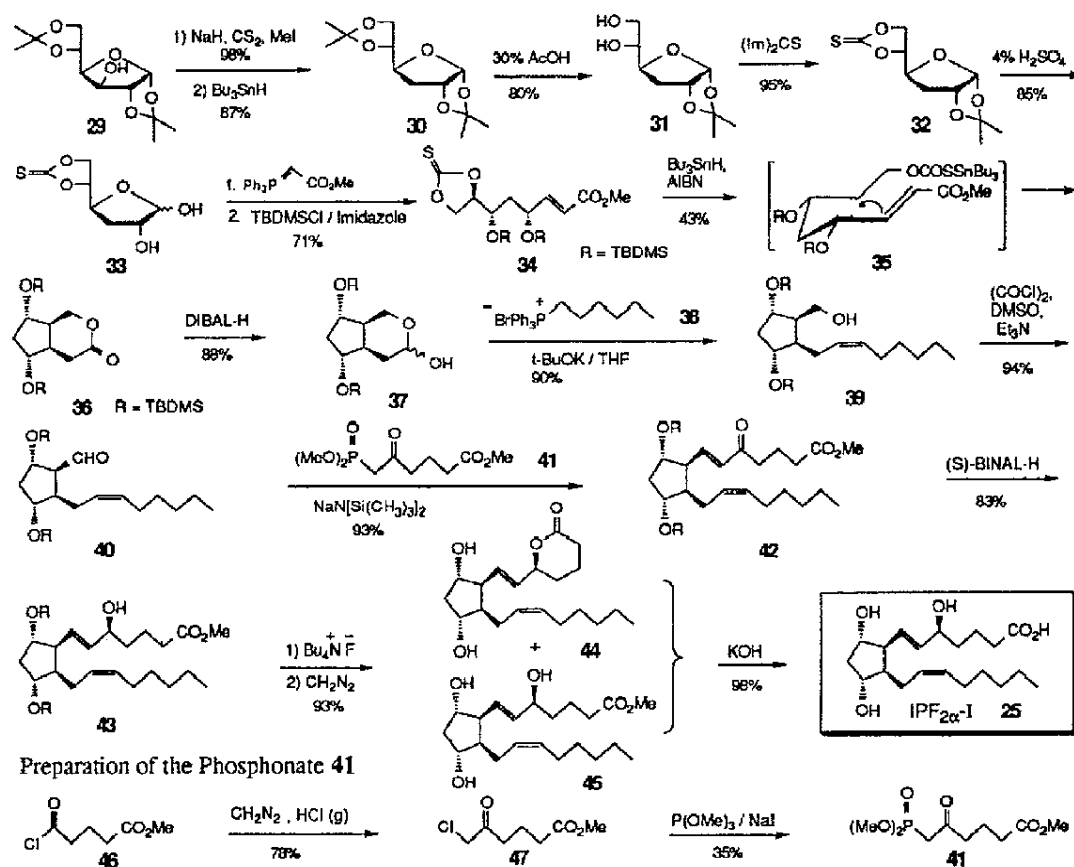


We report here the first proof of the existence *in vivo* of IPF<sub>2α</sub>-I<sup>14</sup> 25, an isoprostane of Type I 9, validating our proposal as to the existence of several classes of isoprostanes. This has been achieved by a two-step strategy involving the first total synthesis of IPF<sub>2α</sub>-I 25 and its identification in human urine.

The synthesis of IPF<sub>2α</sub>-I 25 is shown in Scheme 3. The deoxygenation of diacetone-D-glucose 29 was achieved by first preparing the xanthate derivative in 98% yield using sodium hydride, carbon disulfide and methyl iodide, and then treating it with tri-*n*-butyltin hydride to give 3-deoxydiacetone-D-glucose 30 in 87% yield.<sup>15</sup> The chemoselective cleavage of the 5,6-isopropylidene group in 30 with 30% aqueous acetic acid at room temperature afforded the diol 31 in 80% yield. Treatment of diol 31 with thiocarbonylbis(imidazole) in 1,2-dichloroethane at room temperature gave the thionocarbonate derivative 32 in 95% yield. Removal of the 1,2-isopropylidene group with 4% aqueous sulfuric acid in tetrahydrofuran (THF) at reflux temperature gave the epimeric lactols 33 in 85% yield. The Wittig reaction of 33 with methyl(triphenylphosphoranylidene)acetate in THF was carried out at room temperature and the resulting crude mixture was treated with *t*-butyldimethylsilyl chloride in dimethylformamide at 60 °C to give 34 in 71% yield. Finally, the cyclization of 34 was achieved using tri-*n*-butyltin hydride (2 equiv.) and AIBN (0.5 equiv.) in toluene at reflux temperature to afford the *syn-anti-syn* lactone 36 as the major product in 43% isolated yield.<sup>16</sup> The stereoselective formation of the lactone 36 as the major product is probably due to a preferred chair conformation of radical intermediate 35.

The reduction of lactone **36** with DIBAL-H in methylene chloride at  $-78^{\circ}\text{C}$ , followed by acidic work-up, afforded a mixture of lactol epimers **37** in 88% yield, used as such in the next step. The Wittig reaction with commercial hexyltriphenylphosphonium bromide **38** (4 equiv.) and potassium *t*-butoxide (3.99 equiv.) at  $-78^{\circ}\text{C}$

**Scheme 3.** Synthesis of IPF<sub>2α</sub>-I **25**, (5*S*,6*E*,8*β*,9*α*,11*α*,14*Z*)-5,9,11-trihydroxyprosta-6,14-dien-1-oic acid.



proceeded smoothly to give the *cis* olefin **39** in 90% yield. The Swern oxidation of the alcohol **39** using oxalyl chloride, DMSO, and triethylamine yielded aldehyde **40** in 94% yield. Horner-Emmons reaction of **40** at  $-78^{\circ}\text{C}$ , to introduce the upper side chain using the anion of  $\beta$ -ketophosphonate **41** generated with sodium *bis*(trimethylsilyl)amide in THF at room temperature, afforded the enone **42** in 93% yield. The enantioselective reduction of the C<sub>5</sub> keto group in **42** with chiral reducing agent<sup>17</sup> (S)-BINAL-H proceeded well and afforded the desired pure 5(*S*) derivative **43** in 83% yield. The deprotection of the *bis*-silyl groups in **43** was carried out using tetrabutylammonium fluoride in THF at room temperature and the crude product was treated with diazomethane to give the lactone **44** and the methyl ester **45**. The mixture of **44** and **45** can be separated by flash column chromatography to afford the pure compounds. Finally, the basic hydrolysis of **44** and **45** with aqueous potassium hydroxide in dioxane at room temperature yielded the desired IPF<sub>2α</sub>-I **25** in 98% yield.<sup>18</sup>

A urine sample from normal volunteers was collected and prepared for GC-MS analysis as described

previously.<sup>9</sup> The TBDMS-pentafluorobenzyl ester (PFB) of the urinary sample was prepared, as well as the *tris*-TBDMS-PFB derivative of synthetic IPF<sub>2α</sub>-I, 8-*epi*-PGF<sub>2α</sub>, and <sup>18</sup>O<sub>2</sub> 8-*epi*-PGF<sub>2α</sub>, used as the internal standard. The retention time of the internal control was 19.655 min. The new IPF<sub>2α</sub>-I peak appears at retention time 19.356 min. This peak was identified by comigration with authentic IPF<sub>2α</sub>-I **25**.

In addition, we converted the urinary IPF<sub>2α</sub>-I to its 6-membered-ring lactone and confirmed its identity by comparison with the synthetic lactone **44** in the following manner. We treated the urinary mixture containing IPF<sub>2α</sub>-I with excess dicyclohexylcarbodiimide. The urinary lactone was purified on TLC using the synthetic lactone **44** as visualization standard. The urinary lactone was isolated, hydrolyzed with KOH/water and the reaction mixture acidified, extracted with ethyl acetate and the solvent evaporated. The GC-MS of the *tris*-TBDMS-PFB ester of the residue was identical to the IPF<sub>2α</sub>-I derivative obtained from the synthetic lactone **44** submitted to the same procedure.

Identification of the new isoprostane, IPF<sub>2α</sub>-I **25**, in biological fluids is a significant step in the effort to evaluate the free-radical-initiated biochemical pathway in disease states.

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14. Assumptions we used to name this isoprostane: IP = isoprostane; F<sub>α</sub> = two hydroxyls on the ring with the stereochemistry shown; 2 = two double bonds. The structure as shown provides the basis for naming all isoprostanes of Type I, e.g. the epimer at C<sub>5</sub> in IPF<sub>2α</sub>-I will be called 5-*epi*-IPF<sub>2α</sub>-I.
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18. Spectral data for the IPF<sub>2α</sub>-I **25**: <sup>1</sup>H NMR (CD<sub>3</sub>COCD<sub>3</sub>) δ 5.58-5.48 (m, 2H, C7-H, C6-H), 5.46 (m, 2H, C14-H, C15-H), 4.05 (m, 1H, C5-H), 3.97 (m, 1H, C9-H), 3.88 (m, 1H, C11-H), 2.68 (m, 1H, C8-H), 2.4 (q, J = 7.3 and 14.3 Hz, 1H, C10-H), 2.3 (t, J = 7.3 Hz, C2-H<sub>2</sub>), 2.17-1.95 (m, 5H, C12-H, C3-H<sub>2</sub>, C13-H<sub>2</sub>), 1.78-1.6 (m, 2H, C10-H, C4-H), 1.6-1.48 (m, 3H, C4-H, C16-H<sub>2</sub>), 1.4-1.26 (m, 6H, C17-H<sub>2</sub>, C18-H<sub>2</sub>, C19-H<sub>2</sub>), 0.9 (t, J = 6.8 Hz, 3H, C20-H). <sup>13</sup>C NMR (CD<sub>3</sub>COCD<sub>3</sub>) δ 174.8, 136.7, 130.9, 129.8, 129.6, 76.2, 76.1, 72.4, 53.7, 51.5, 44.0, 37.9, 34.2, 32.3, 30.2, 29.3, 27.2, 23.3, 22.0, 14.4. HRFAB MS *m/z* calc for (M+Na)<sup>+</sup> 377.2304, found 377.2293.

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